



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification: A61L 25/00, A61K 35/14	A1	(11) International Publication Number: WO 00/62828 (43) International Publication Date: 26 October 2000 (26.10.2000)
(21) International Application Number: PCT/US99/08550 (22) International Filing Date: 19 April 1999 (19.04.1999) (30) Priority Data: 09/063,338 20 April 1998 (20.04.1998) US (60) Parent Application or Grant MEDTRONIC, INC. [/]; O. BAUGH, Robert, F. [/]; O. LIM, Lisa, M. [/]; O. JOHNSTON, Julie, S. [/]; O. RIVERA, John, G. [/]; O. PETERSEN, Steven, C. ; O.		Published

(54) Title: AUTOLOGOUS FIBRIN SEALANT AND METHOD FOR MAKING THE SAME
(54) Titre: FIBRINE AUTOLOGUE D'OBTURATION ET SES METHODES DE FABRICATION

(57) Abstract

In general, the present invention relates to a two-phase method for forming an autologous bioadhesive sealant composition or fibrin glue wherein all of the blood components for the bioadhesive sealant are derived from a patient to whom the bioadhesive sealant will be applied. First, a platelet rich plasma and a platelet poor plasma are formed by centrifuging a quantity of anticoagulated whole blood that was previously drawn from the patient. The platelet rich plasma and platelet poor plasma are then divided into two portions. To the first portion, which is used in phase-one, a compound that reverses the effect of the anticoagulant is added, and a clot is allowed to form. The clot is then triturated and the resulting serum, containing autologous thrombin, is collected. The serum obtained from phase-one is then mixed with the second portion of the platelet rich plasma or platelet poor plasma, used in phase-two, to form the bioadhesive sealant of the present invention.

(57) Abrégé

En général, la présente invention porte sur une méthode à deux phases conçue pour préparer une composition d'obturation bioadhésive ou une colle à base de fibrine dans laquelle tous les composants du sang pour la composition d'obturation bioadhésive proviennent du patient auquel ladite composition bioadhésive sera administrée. Premièrement, un plasma riche en plaquettes et un plasma pauvre en plaquettes se forment par centrifugation d'une quantité de sang entier additionné d'anticoagulant qui a préalablement été prélevée chez un patient. Le plasma riche en plaquettes et le plasma pauvre en plaquettes sont ensuite divisés en deux fractions. Un composé est ajouté à la première fraction, utilisée lors de la première phase, pour inverser les effets de l'anticoagulant et pour permettre la formation d'un caillot. Le caillot est ensuite trituré et le sérum qui en résulte, contenant un autologue à base de thrombine, est récolté. Le sérum obtenu au cours de la première phase est ensuite mélangé à la seconde fraction de plasma riche en plaquettes ou de plasma pauvre en plaquettes, utilisée dans la seconde phase, pour préparer la composition d'obturation bioadhésive de la présente invention.

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(21) International Application Number: PCT/US99/08550 (22) International Filing Date: 19 April 1999 (19.04.99) (30) Priority Data: 09/063,338 20 April 1998 (20.04.98) US (71) Applicant: MEDTRONIC, INC. [US/US]; 7000 Central Avenue, Minneapolis, MN 55432 (US). (72) Inventors: BAUGH, Robert, F.; 7926 East Windcrest Row, Parker, CO 80134 (US). LIM, Lisa, M.; 20472 E. Mansfield Place, Aurora, CO 80013 (US). JOHNSTON, Julie, S.; 6274 Red Canyon Drive, Apt. D, Highlands, CO 80126 (US). RIVERA, John, G.; 15191 E. Layton Place, Aurora, CO 80015 (US). (74) Agents: PETERSEN, Steven, C. et al.; Chrisman, Bynum & Johnson, P.C., 1900 Fifteenth Street, Boulder, CO 80302 (US).			(81) Designated States: DE, JP. Published With international search report.
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Description

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**AUTOLOGOUS FIBRIN SEALANT AND
METHOD FOR MAKING THE SAME**

Description

Cross-Reference to Related Patent Applications

This patent application is a continuation-in-part of U.S. Patent Application No. 08/640,278, filed April 30, 1996, and entitled Method For Making Autologous Fibrin Sealant.

Technical Field

The present invention relates to autologous bioadhesive sealant compositions, and more particularly to a convenient and practical two-phase method for preparing a bioadhesive sealant specifically from blood components derived from the patient who is to receive the bioadhesive sealant.

Background Art

When the lining of a blood vessel is damaged, a complex series of events takes place which is designed to prevent blood loss and, ultimately, to restore the integrity of the vessel. Although short-lived vasoconstriction and physical factors such as the pressure of extruded blood on the vessel wall may play some part in haemostasis, the main factors in the haemostatic mechanism are platelets and the blood coagulation system.

Blood coagulation is the result of the complex interaction of a number of protein clotting factors through a cascade, see Figure 1. In general, damage to the vascular endothelium exposes subendothelial structures which attract platelets and induce them to aggregate reversibly. The protein thrombin, formed during activation of the coagulation pathway generates insoluble cross-linked fibrils of the protein fibrin and causes the platelets to aggregate irreversibly. The resulting platelet-fibrin clot is an effective barrier against loss of blood from the vascular system and also serves as a scaffold for subsequent repair of the lining of the blood vessel.

A bioadhesive sealant or fibrin glue is a relatively new technological advance which duplicates the biological process of the final stage of blood coagulation. Clinical reports document the utility of fibrin glue in a variety of surgical fields, such as, cardiovascular, thoracic, transplantation, head and neck, oral, gastrointestinal, orthopedic, neurosurgical, and plastic surgery. At the time of surgery, the two primary components comprising the fibrin glue, fibrinogen and thrombin, are mixed together to form a clot. The clot adheres to the necessary tissues, bone, or nerve within seconds, but is then slowly reabsorbed by the body in approximately 10 days by fibrinolysis. Important features of fibrin glue is its ability to: (1) achieve haemostasis at vascular anastomoses particularly in areas which are difficult to approach with sutures or where suture placement presents

5 excessive risk; (2) control bleeding from needle holes or arterial tears which cannot be controlled by
suturing alone; and (3) obtain haemostasis in heparinized patients or those with coagulopathy. See,
Borst, H.G., *et al.*, *J. Thorac. Cardiovasc. Surg.*, 84:548-553 (1982); Walterbusch, G. J., *et al.*,
10 *Thorac. Cardiovasc. Surg.*, 30:234-235 (1982); and Wolner, F. J., *et al.*, *Thorac. Cardiovasc. Surg.*,
5 30:236-237 (1982).

Despite the effectiveness and successful use of fibrin glue by medical practitioners in
Europe, neither fibrin glue nor its essential components fibrinogen and thrombin are widely used in
15 the United States. In large part, this stems from the 1978 U.S. Food and Drug Administration ban on
the sale of commercially prepared fibrinogen concentrate made from pooled donors because of the
10 risk of transmission of viral infection, in particular the hepatitis-causing viruses such as HBV and
HCV (also known as non A, non B hepatitis virus). In addition, the more recent appearance of other
20 lipid-enveloped viruses such as HIV, associated with AIDS, cytomegalovirus (CMV), as well as
Epstein-Barr virus, and the herpes simplex viruses in fibrinogen preparations make it unlikely that
there will be a change in this policy in the foreseeable future. For similar reasons, human thrombin is
25 also not currently authorized for human use in the United States. Bovine thrombin, which is licensed
15 for human use in the United States is obtained from bovine sources which do not appear to carry
significant risks for HIV and hepatitis, although other bovine pathogens, such as bovine spongiform
encephalitis, may be present.

There have been a variety of methods developed for preparing fibrin glue. For example,
30 Rose, *et al.* discloses a method of preparing a cryoprecipitated suspension containing fibrinogen and
20 Factor XIII useful as a precursor in the preparation of a fibrin glue which involves (a) freezing fresh
frozen plasma from a single donor such as a human or other animal, e.g. a cow, sheep or pig, which
35 has been screened for blood transmitted diseases, e.g. one or more of syphilis, hepatitis or acquired
immune deficiency syndrome at about 80° C. for at least about 6 hours, preferably for at least about
25 12 hours; (b) raising the temperature of the frozen plasma, e.g. to between about 0° C. and room
temperature, so as to form a supernatant and a cryoprecipitated suspension containing fibrinogen and
40 Factor XIII; and (c) recovering the cryoprecipitated suspension. The fibrin glue is then prepared by
applying a defined volume of the cryoprecipitate suspension described above and applying a
composition containing a sufficient amount of thrombin, e.g. human, bovine, ovine or porcine
45 30 thrombin, to the site so as to cause the fibrinogen in the suspension to be converted to the fibrin glue
which then solidifies in the form of a gel. See U.S. Patent Number 4,627,879.

A second technique for preparing fibrin glue is disclosed by Marx in his U.S. Patent Number
5,607,694. Essentially a cryoprecipitate as discussed previously serves as the source of the
50 fibrinogen component and then Marx adds thrombin and liposomes. A third method discussed by

5 Berruyer, M. *et al.*, entitled "Immunization by bovine thrombin used with fibrin glue during
cardiovascular operations," (*J. Thorac. Cardiovasc. Surg.*, 105(5):892-897 (1992)) discloses a fibrin
glue prepared by mixing bovine thrombin not only with human coagulant proteins, such as
10 fibrinogen, fibronectin, Factor XIII, and plasminogen, but also with bovine aprotinin and calcium
chloride.

5 The above patents by Rose, *et al.*, and Marx, and the technical paper by Berruyer, *et al.* each
disclose methods for preparing fibrin sealants; however, each of these methods suffer disadvantages
15 associated with the use of bovine thrombin as the activating agent. A serious and life threatening
consequence associated with the use of fibrin glues comprising bovine thrombin is that patients have
20 been reported to have a bleeding diathesis after receiving topical bovine thrombin. This complication
occurs when patients develop antibodies to the bovine factor V in the relatively impure bovine
thrombin preparations. These antibodies cross-react with human factor V, thereby causing a factor V
deficiency that can be sufficiently severe to induce bleeding and even death. See, Rapaport, S.I., *et*
25 *al.*, *Am. J. Clin. Pathol.*, 97:84-91 (1992); Berruyer, M., *et al.*, *J. Thorac. Cardiovasc. Surg.*,
105:892-897 (1993); Zehnder, J., *et al.*, *Blood*, 76(10):2011-2016 (1990); Muntean, W., *et al.*, *Acta*
Paediatr., 83:84-7 (1994); Christine, R.J., *et al.*, *Surgery*, 127:708-710 (1997).

Further disadvantages associated with the methods disclosed by Marx and Rose, *et al.* are
that the cryoprecipitate preparations require a large time and monetary commitment to prepare.
30 Furthermore, great care must be taken to assure the absence of any viral contaminants.

20 A final disadvantage associated with the methods previously disclosed is that while human
thrombin is contemplated for use as an activator, human thrombin is not available for clinical use and
there is no evidence that patients will not have an antigenic response to human thrombin. By
35 analogy, recombinant human factor VIII has been shown to produce antigenic responses in
hemophiliacs. See, Biasi, R. de., *Thrombosis and Haemostasis*, 71(5):544-547 (1994).
25 Consequently, until more clinical studies are performed on the effect of human recombinant thrombin
one cannot merely assume that the use of recombinant human thrombin would obviate the antigenic
40 problems associated with bovine thrombin. A second difficulty with thrombin is that it is
autocatalytic, that is, it tends to self destruct making handling and prolonged storage a problem.

There is still a need, therefore, for a convenient and practical method for preparing a
45 30 bioadhesive sealant composition wherein the resulting bioadhesive sealant poses a zero risk of
disease transmission and a zero risk of causing an adverse physiological reaction.

Disclosure of Invention

Accordingly, it is an object of this invention to provide a method for preparing a completely
50 autologous bioadhesive sealant composition.

Another object of the present invention is to provide an autologous bioadhesive sealant composition wherein the risks associated with the use of bovine and recombinant human thrombin are eliminated.

Additional objects, advantages and novel features of this invention shall be set forth in part in the description that follows, and in part will become apparent to those skilled in the art upon examination of the following specification or may be learned by the practice of the invention. The objects and advantages of the invention may be realized and attained by means of the instrumentalities, combinations, compositions, and methods particularly pointed out in the appended claims.

To achieve the foregoing and other objects and in accordance with the purposes of the present invention, as embodied and broadly described therein, the method of this invention comprises the formation of an autologous bioadhesive sealant comprising the steps of forming a platelet rich plasma or platelet poor plasma containing an anticoagulant. The platelet rich plasma or platelet poor plasma is then divided into two portions and the first portion is restored so that it can coagulate thus forming a clot. The clot is then triturated and the resulting serum is collected. The bioadhesive sealant composition is then prepared by combining a defined volume of the second portion of platelet rich plasma or platelet poor plasma with a sufficient volume of serum causing the fibrinogen in the second portion of platelet rich plasma or platelet poor plasma to be converted to fibrin which then solidifies in the form of a gel.

Brief Description of the Drawings

The accompanying drawings, which are incorporated herein and form a part of the specification illustrate preferred embodiments of the present invention, and together with the description, serve to explain the principles of the invention.

In The Drawings:

Figure 1 is a diagrammatic representation of the blood coagulation cascade.

Figure 2a is a flow diagram representing the initial portion of the method of the present invention used to obtain platelet rich plasma and platelet poor plasma.

Figure 2b is a flow diagram representing the final portion of the method for preparing the bioadhesive sealant composition of the present invention using platelet rich plasma as a starting material.

Figure 2c is a flow diagram representing the final portion of the method for preparing the bioadhesive sealant composition of the present invention using platelet poor plasma as a starting material.

Figure 3 is a graphic representation of the effect that the serum-to-plasma ratio has on

5 clotting times.

Figure 4a is a graphic representation of the relationship between clotting time and actual gel time using blood drawn from a donor.

10 Figure 4b is a graphic representation of the relationship between clotting time and actual gel time using blood drawn from a donor.

5 Figure 5a graphically represents the effect of calcium addition on clotting times and gel times using blood drawn from a donor.

15 Figure 5b graphically represents the effect of calcium addition on clotting times and gel times using blood drawn from a donor.

10 Figure 6 graphically represents the effect of calcium addition on the clotting times of platelet rich plasma and platelet poor plasma.

20 **Best Mode for Carrying out the Invention**

In general, the present invention relates to a two-phase method, shown in Figures 2a, 2b and 2c, for forming an autologous bioadhesive sealant composition or fibrin glue wherein all of the blood components for the bioadhesive sealant are derived from a patient to whom the bioadhesive sealant will be applied. First, a platelet rich plasma and a platelet poor plasma are formed by centrifuging a quantity of anticoagulated whole blood that was previously drawn from the patient. The platelet rich plasma and platelet poor plasma are then divided into two portions. To the first portion, which is used in phase-one, a compound that reverses the effect of the anticoagulant is added, and a clot is allowed to form. The clot is then triturated and the resulting serum, containing autologous thrombin, is collected. The serum obtained from phase-one is then mixed with the second portion of the platelet rich plasma or platelet poor plasma, used in phase-two, to form the bioadhesive sealant of the present invention.

25 The method of the present invention for preparing an autologous bioadhesive composition, discussed in further detail below, is represented in the flow diagram depicted in Figures 2a, 2b and 2c. The method of the present invention begins by forming anticoagulated whole blood 90 which is achieved by collecting a patient's whole blood 80 in a medium containing an anticoagulation agent, such as sodium citrate (citrate) or heparin. The act of drawing blood initiates clotting reactions, and unless something is done to stop the process, a clot will form. The formation of a clot is a multi-step process and several of these steps require the presence of calcium ions. By removing the calcium ions present in whole blood, as is the effect when the blood is collected in citrate, the blood can be prevented from clotting. To reinitiate the clot-forming process, calcium is added back into the whole blood (recalcification). A calcium chelating agent is a chemical which reacts with the calcium, present in blood, in such a fashion that the calcium can no longer function in blood coagulation. The

most common chelating agent is a salt of citric acid (citrate), since it has the fewest side effects on the components of the clotting system. By collecting blood into a medium containing a calcium chelating agent such as citric acid, sample collection and further preparations of the citrated sample can be performed over a time period of up to several hours.

Preferably, the whole blood 80 is collected and mixed with a 3.8% solution of sodium citrate (referred to herein as "citrate collection medium") specifically in a 9:1 ratio of blood to citrate collection medium. A 3.8% solution of sodium citrate is prepared by adding 3.8 grams of sodium citrate per 100 ml of water. While a 3.8% sodium citrate collection medium is that which is frequently used to collect and preserve blood, the person skilled in this art will recognize that the ratio of sodium citrate to whole blood could be in the range of about 10.9 - 12.9% mM/L, final concentration.

The anticoagulated whole blood 90 is next centrifuged at a rate of approximately 20 - 50 r.c.f.'s (relative centrifugal force) for 10 - 40 minutes, and preferably in a refrigerated centrifuge at 25 r.c.f.'s for 20 minutes, resulting in the formation of two liquid phases. The top phase is a platelet rich plasma (PRP) 100, and the bottom phase is anticoagulated whole blood minus the platelet rich plasma 190. The platelet rich plasma (PRP) 100 is then gently drawn off and saved in a container. The remaining anticoagulated whole blood minus the platelet rich plasma phase 190 is further centrifuged at a rate of approximately 3000 - 4500 r.c.f.'s for 15 - 30 minutes, and preferably in a refrigerated centrifuge at 3850 r.c.f.'s for 20 minutes. This higher rate of centrifugation results in the red blood cells, white blood cells and platelets being spun out of the anticoagulated whole blood minus the platelet rich plasma phase 190 thereby forming a pellet (not shown) comprising cellular components which is to be discarded. The resulting platelet poor plasma (PPP) 200 is then decanted from the pellet and saved in a container. The containers (not shown) which store the platelet rich plasma 100 and platelet poor plasma 200 may have either wettable surfaces (such as, silica, diatomaceous earth, koalin, etc.) or non-wettable surfaces (such as plastic, siliconized glass, etc.). Since surfaces play a role in activating blood coagulation, the surface of the container chosen to store either the platelet rich plasma 100 or the platelet poor plasma 200 is dependent on whether clot formation is desired quickly or slowly. Chemical activators, such as koalin, can also be used to speed up the clotting time; however, their subsequent removal would also be necessary. In the preferred embodiment, a glass tube is the preferred container used to collect the platelet rich plasma 100 and the platelet poor plasma 200.

In the preferred embodiment, according to route 101, the platelet rich plasma 100 is divided into two portions. The first portion is approximately $\frac{1}{4}$ to $\frac{1}{2}$ of the total volume of platelet rich plasma 100 and is utilized in phase-one to prepare the thrombin, while the second portion of platelet

5 rich plasma 100 is utilized in phase-two. Once the platelet rich plasma 100 and the platelet poor plasma 200 are obtained, the preferred methods to obtain the bioadhesive sealant compositions in an expedited manner, that is, in less than two minutes, are detailed diagrammatically in routes 101 or 10 201, shown in Figures 2b and 2c, respectively and discussed in detail below. If, however, a longer clotting time, that is, in a range of two to eight minutes, is desirous the method to obtain the bioadhesive sealant composition of the present invention can proceed along the routes 105 and 205, which are also detailed diagrammatically in Figures 2b and 2c, respectively and discussed in detail below.

15 Phase-one according to the preferred embodiment begins by restoring the clot-forming process. To accomplish this, an agent (restoration agent) capable of reversing the effects of the 10 anticoagulation agent is added back into the first portion of the platelet rich plasma 100. In the presently preferred embodiment of the invention, the reversal of the anticoagulant is accomplished using calcium chloride. However, any substance which is known or found to be functionally 20 equivalent to calcium chloride, such as, calcium gluconate, in restoring the coagulation activity of citrated blood may be used in the practice of the present invention. Thus, although calcium chloride 25 is the presently preferred calcium salt for use in the invention, any calcium salt which functions in a similar manner to calcium chloride may be used in the invention. Similarly, although many blood coagulation reactions are currently believed to require calcium ions as cofactors, any substance which 30 is known or subsequently found to be functionally equivalent to calcium in facilitating these coagulation reactions may be used, either individually or in combination with calcium, in the practice 20 of the present invention. If the anticoagulation agent used was heparin, then heparinase would be used to reverse the effect of the anticoagulation agent. The concentration of the restoration agent used to reverse the anticoagulation will depend in part, upon the concentration of the anticoagulation agent in 35 the platelet rich plasma 100 and the stoichiometry of the chelating and coagulation reactions. However, the concentration of the restoration agent used to reverse the anticoagulation must be 25 sufficient to achieve clot formation.

40 Upon restoration of the platelet rich plasma 100 as shown in Figure 2b, a clot 110 will naturally form. The resulting clot 110 is then triturated by high speed centrifugation, or squeezed through a mesh, thus releasing a serum 120 that comprises thrombin. In the preferred embodiment, 45 30 the serum 120 is then mixed with the second portion of platelet rich plasma (PRP) 100 to form the bioadhesive sealant composition 130 of the present invention in less than two minutes and in quantities sufficient for clinical use.

50 In an alternative embodiment, serum 120 is mixed with the platelet poor plasma 200 of phase-two thereby forming the autologous bioadhesive sealant composition 140 of the present

invention in less than two minutes.

A third embodiment of the present invention, route 105, shown in Figure 2b, contemplates collecting the original quantity of platelet rich plasma (PRP) 100 derived from the anticoagulated whole blood 90 in a container, having a wettable surface, such as glass. The platelet rich plasma 100 is then recalcified and the bioadhesive sealant composition 150 forms. The desired bioadhesive sealant composition 150 will require approximately two to eight minutes to form as opposed to less than a two minute formation as was described in the preferred embodiment.

In the fourth embodiment depicted diagrammatically by route 201 in Figure 2c, the platelet poor plasma 200, rather than the platelet rich plasma 100, is divided into two portions, as discussed previously in the preferred embodiment. The first portion, used in phase-one, which is approximately $\frac{1}{4}$ to $\frac{1}{2}$ the original volume is stored in a container having a wettable surface, then the restoration agent, preferably calcium chloride, is added directly to the platelet poor plasma 200. Surface activation of the restored platelet poor plasma 200 occurs as result of the container's surface and a clot forms. The resulting clot is triturated, as described previously, and the serum 220 is collected. Serum 220 is then mixed with the platelet rich plasma 100 of phase-two thereby forming the autologous bioadhesive sealant composition 230.

In the fifth embodiment, serum 220 is mixed with the platelet poor plasma 200 of phase-two thereby forming the bioadhesive sealant composition 240 in less than two minutes.

The sixth embodiment follows route 205, shown in Figure 2c wherein the original quantity of platelet poor plasma 200, derived from the anticoagulated whole blood minus platelet rich plasma 190, is collected in a container having a wettable surface, such as glass. The platelet poor plasma 200 is then recalcified and the bioadhesive sealant composition 250 forms.

A seventh embodiment contemplates mixing human recombinant thromboplastin directly with the platelet rich plasma to form a bioadhesive sealant composition (not shown). Alternatively, human recombinant thromboplastin is utilized to generate thrombin in a small aliquot of plasma and then the resulting thrombin is combined with the platelet rich plasma to form a bioadhesive sealant. Thromboplastin may be later removed by centrifugation.

The tensile strength of the bioadhesive sealant compositions of the present invention can be effected by the addition of calcium ions. Consequently, if a stronger bioadhesive sealant composition is desired using the methods discussed above and disclosed in routes 101 and 201, in Figures 2b and 2c, respectively, more calcium ions may be added at the time the serum is introduced into the platelet rich plasma 100 or the platelet poor plasma 200. Alternatively, if the method of preparing the bioadhesive sealant compositions follows routes 105 and 205, depicted in Figures 2b and 2c, respectively, then calcium ions may be introduced directly into the platelet rich plasma 100 or the

5 platelet poor plasma 200 and the bioadhesive sealant compositions 150 and 250, respectively, will form.

10 As discussed in further detail below, the time period necessary for the formation of the bioadhesive sealant composition of the present invention is dependent on the quantity of serum added. A 1:4, 1:2 and 3:4 ratio of serum to platelet rich plasma or platelet poor plasma results in the formation of the bioadhesive gel composition in approximately 90, 55 and 30 seconds, respectively. Furthermore, due to the fact that thrombin is autocatalytic, it is important that the serum be used 15 within five hours of preparation, preferably within two hours and ideally immediately. Alternatively, the serum can be chilled or frozen indefinitely.

10 The bioadhesive sealant compositions of this invention may be used for sealing a surgical wound by applying to the wound a suitable amount platelet rich plasma or platelet poor plasma once it has begun to gel. Moreover, due to the fact that the bioadhesive sealant compositions of the present invention have been prepared solely from blood components derived from the patient that is to receive the bioadhesive sealant there is a zero probability of introducing a new blood transmitted 25 disease to the patient. The methods of the present invention may be further modified so that the formed bioadhesive sealant composition functions not only as a haemostatic agent, but also as an adjunct to wound healing and as a matrix for delivery of drugs and proteins with other biologic activities. For example, it is well known that fibrin glue has a great affinity to bind bone fragments which is useful in bone reconstruction, as in plastic surgery or the repair of major bone breaks. 30 Consequently, in keeping with the autologous nature of the bioadhesive sealant composition of the present invention autologous bone from a patient can be ground or made into powder or the like, and mixed into the platelet rich plasma obtained in phase-two of the methods of the present invention. Serum comprising thrombin is then mixed in with the platelet rich plasma and bone fragments in an amount sufficient to allow the resulting gel to be applied to the desired locale where it congeals. 35

25 In instances where the desired bioadhesive sealant composition of the present invention is to further function as a delivery device of drugs and proteins with other biologic activities the method of the present invention may be modified as follows. Prior to adding the serum comprising thrombin, obtained in phase-one, to the platelet rich plasma of phase-two a wide variety of drugs and proteins with other biologic activities may be added to the platelet rich plasma of phase-two. Examples of the agents to be added to the platelet rich plasma prior to the addition of the serum include, but are not 40 limited to, analgesic compounds, antibacterial compounds, including bacteriocidal and bacteriostatic compounds, antibiotics (e.g., adriamycin, erythromycin, gentimycin, penicillin, tobramycin), antifungal compounds, anti-inflammatories, antiparasitic compounds, antiviral compounds, enzymes, 45 enzyme inhibitors, glycoproteins, growth factors (e.g. lymphokines, cytokines), hormones, steroids, 50

5 glucocorticosteroids, immunomodulators, immunoglobulins, minerals, neuroleptics, proteins,
peptides, lipoproteins, tumoricidal compounds, tumorstatic compounds, toxins and vitamins (e.g.,
Vitamin A, Vitamin E, Vitamin B, Vitamin C, Vitamin D, or derivatives thereof). It is also
10 envisioned that selected fragments, portions, derivatives, or analogues of some or all of the above
5 may be used.

A number of different medical apparatuses and testing methods exist for measuring and
determining coagulation and coagulation-related activities of blood. These apparatuses and methods
15 can be used to assist in determining the optimal formulation of activator, that is, thrombin, calcium
and plasma necessary to form the bioadhesive sealant composition of the present invention. Some of
10 the more successful techniques of evaluating blood clotting and coagulation are the plunger
techniques illustrated by U.S. Pat. Nos. 4,599,219 to Cooper et al., 4,752,449 to Jackson et al., and
20 5,174,961 to Smith, all of which are assigned to the assignee of the present invention, and all of
which are incorporated herein by reference.

Automated apparatuses employing the plunger technique for measuring and detecting
15 coagulation and coagulation-related activities generally comprise a plunger sensor cartridge or
cartridges and a microprocessor controlled apparatus into which the cartridge is inserted. The
apparatus acts upon the cartridge and the blood sample placed therein to induce and detect the
coagulation-related event. The cartridge includes a plurality of test cells, each of which is defined by
30 a tube-like member having an upper reaction chamber where a plunger assembly is located and where
20 the analytical test is carried out, and a reagent chamber which contains a reagent or reagents. For an
activated clotting time (ACT) test, for example, the reagents include an activation reagent to activate
coagulation of the blood. A plug member seals the bottom of a reagent chamber. When the test
35 commences, the contents of the reagent chamber are forced into the reaction chamber to be mixed
with the sample of fluid, usually human blood or its components. An actuator, which is a part of the
25 apparatus, lifts the plunger assembly and lowers it, thereby reciprocating the plunger assembly
through the pool of fluid in the reaction chamber. The plunger assembly descends by the force of
40 gravity, resisted by a property of the fluid in the reaction chamber, such as its viscosity. When the
property of the sample changes in a predetermined manner as a result of the onset or occurrence of a
coagulation-related activity, the descent rate of the plunger assembly therethrough is changed. Upon
45 30 a sufficient change in the descent rate, the coagulation-related activity is detected and indicated by
the apparatus.

Using the methods discussed above, cartridges were assembled with serum obtained from
either platelet rich plasma or platelet poor plasma, and CaCl_2 in the reagent chambers. Clotting time
50 tests were performed by the automated process with either platelet rich plasma (PRP) or platelet poor

5 plasma (PPP) dispersed into the reaction chambers of the cartridges. In the first experiment, the results of which are represented in Figure 3, the amount of serum, the type of plasma from which the serum was derived, and the type of plasma the serum was mixed with were tested to determine the shortest clotting times. The ratios of serum to platelet rich plasma or platelet poor plasma that were
10 studied included 1:4, 1:2, and 3:4. In the second set of experiments, the results of which are represented in Figures 4a and 4b, the relationship between actual gel time for the bioadhesive sealant composition of the present was compared to the clotting time in the cartridge, wherein there is a 0, 30, or 60 minute delay of adding the serum from its generation. The third set of experiments, the results of which are represented in Figures 5a and 5b, studied the effect of calcium addition on actual
15 gel time versus clotting time in the cartridge. The final set of experiments, the results of which are represented in Figure 6, studied the effect of adding calcium on clotting times.

20 Although clotting times varied among donors, comparisons of clotting times for individual donors show significant effects of the serum to plasma ratio and the calcium concentration. For all donors, the shortest clotting times occurred for the 3:4 ratio, with clotting times that were 47%
25 shorter than those for the 1:4 ratio. Although the difference in clotting times for the 3:4 ratio and the 1:2 ratio was not statistically significant, the clotting times were consistently shorter using the 3:4 ratio for all donors. These results demonstrate that clotting times may be shortened by increasing the serum to platelet rich plasma ratio. Similarly, clotting times were significantly affected by the
30 amount of calcium added, with the shortest clotting times obtained when no calcium was added, suggesting that the serum contained levels of calcium that were sufficient to recalcify the citrated platelet rich plasma. Preliminary results from the scale-up experiments suggest that experimental clotting times in the cartridges correlate with actual gel times.

35 The invention is further illustrated by the following non-limited examples. All scientific and technical terms have the meanings as understood by one with ordinary skill in the art. The specific
40 examples which follow illustrate the methods in which the bioadhesive sealant compositions of the present invention may be prepared in a clinical setting and are not to be construed as limiting the invention in sphere or scope. The methods may be adapted to variation in order to produce compositions embraced by this invention but not specifically disclosed. Further, variations of the methods to produce the same compositions in somewhat different fashion will be evident to one
45 skilled in the art.

EXAMPLES

The examples herein are meant to exemplify the various aspects of carrying out the invention and are not intended to limit the invention in any way.

Example 1**Preparation of Bioadhesive Sealant Composition Using Platelet Rich Plasma and Serum**

10 cc's of platelet rich plasma is added to a sterile glass tube containing .33 cc's of 10% calcium chloride. A stopper is placed in the tube and the contents are gently mixed and the tube is set aside. Gelling of the contents will occur in two to eight minutes. The gel is passed to a dry sterile cup where it is squeezed out 4-6 cc's of the serum, produced therefrom, and 1 cc of air is drawn into a syringe containing 4 cc of platelet rich plasma along with 1 cc of the serum. The bioadhesive sealant composition will gel inside the syringe within approximately two minutes. The gel time may be decreased by increasing the amount of serum added to the platelet rich plasma.

Example 2

10 cc's of whole blood is withdrawn from a patient, placed into a sterile glass tube, and allowed to clot naturally. The clot may be subsequently squeezed or centrifuged to release approximately 4-6 cc's of serum.

An equivalent volume of 10% calcium chloride is then mixed with the serum. 1 cc of this calcified serum is then mixed with 7-8 cc's of platelet rich plasma and 2 cc's of air. The resulting bioadhesive sealant will gel in approximately 1-2 minutes.

Example 3

To a sterile glass syringe containing .33 cc's of 10% calcium chloride, 10 cc's of platelet rich plasma is added. The mixture is allowed to rest for 2 to 8 minutes. Once the gel appears to be appropriately viscous, it may be applied to the wound site.

The techniques demonstrated in examples 1 and 2 may be simultaneously prepared for (a) an additional source of serum; and (b) to confirm successful coagulation.

The foregoing description is considered as illustrative only of the principles of the invention. Furthermore, since numerous modifications and changes will readily occur to those skilled in the art, it is not desired to limit the invention to the exact construction and processes shown as described above. Accordingly, all suitable modifications and equivalents may be resorted to falling within the scope of the invention as defined by the claims which follow.

Claims

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Claims

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. A method of producing an autologous bioadhesive sealant from a single whole blood sample, comprising:

forming an inactive platelet rich plasma from said whole blood sample;
dividing said inactive platelet rich plasma into a first and a second portion;
reactivating said first portion of said inactive platelet rich plasma to form a clot;
tritulating said clot to obtain a serum comprising autologous thrombin; and
mixing said serum with said second portion of said inactive platelet rich plasma.

2. The method of claim 1, wherein said platelet rich plasma contains sodium citrate.

3. The method of claim 1, wherein said inactivated platelet rich plasma contains heparin.

4. The method of claim 2, wherein said reactivation occurs with the addition of calcium ions.

5. The method of claim 3, wherein reactivation occurs with the addition of heparinase.

6. The method of claim 2, wherein said platelet rich plasma containing sodium citrate is formed by:

collecting said whole blood in a 9:1 ratio of whole blood to 3.8% sodium citrate solution forming a citrated blood mixture; and
centrifuging said citrated blood mixture to form a platelet rich plasma containing sodium citrate.

7. The method of claim 1, wherein said platelet rich plasma is obtained from an individual to whom said autologous bioadhesive sealant is to be applied.

8. A fibrin sealant, comprising:
human recombinant thromboplastin; and
platelet rich plasma.

9. The fibrin sealant of claim 8, wherein said platelet rich plasma is isolated from an individual to whom the fibrin sealant is to be applied.

10. A fibrin sealant, comprising:
platelet rich plasma, wherein said platelet rich plasma is isolated from an individual to whom the fibrin sealant is to be applied; and
thrombin, wherein said thrombin is isolated from a portion of said platelet rich plasma by reactivating said portion of said platelet rich plasma to form a clot and tritulating said clot

5 to form a serum containing said thrombin.

11. The method of claim 1, wherein in said triturating comprises squeezing said clot through a mesh to separate said clot from said serum.

10 12. The method of claim 1, wherein said triturating comprises high speed centrifugation to separate said clot from said serum

13. The method of claim 1, further comprising adding calcium ions during said mixing of said serum and said second portion of said platelet rich plasma.

15 14. The method of claim 1, wherein said serum and said second portion of said platelet rich plasma are mixed in a ratio of 1:4 serum to platelet rich plasma.

10 15. The method of claim 1, wherein said serum and said second portion of said platelet rich plasma are mixed in a ratio of 1:2 serum to platelet rich plasma.

20 16. The method of claim 1, wherein said serum and said second portion of said platelet rich plasma are mixed in a ratio of 3:4 serum to platelet rich plasma.

15 17. A method of producing an autologous bioadhesive sealant from a single whole blood sample, comprising:

25 forming an inactive platelet rich plasma and an inactive platelet poor plasma from said whole blood sample;

reactivating said inactive platelet rich plasma to form a clot;

30 triturating said clot to obtain a serum comprising autologous thrombin; and

20 mixing said serum with said platelet poor plasma.

18. The method of claim 17, wherein said platelet rich plasma and said platelet poor plasma each contains sodium citrate.

35 19. The method of claim 18, wherein said reactivation occurs with the addition of calcium ions.

25 20. The method of claim 17, wherein said platelet rich plasma and said platelet poor plasma each contains heparin.

40 21. The method of claim 20, wherein said reactivation occurs with the addition of heparinase.

45 22. The method of claim 18, wherein said platelet rich plasma and said platelet poor plasma containing sodium citrate are formed by:

30 collecting said whole blood in a 9:1 ratio of whole blood to 3.8% sodium citrate solution forming a citrated blood mixture; and

50 centrifuging said citrated blood mixture to form a platelet rich plasma and a platelet poor plasma each containing sodium citrate.

5 23. The method of claim 17, wherein said whole blood sample is obtained from an individual to whom said autologous bioadhesive sealant is to be applied.

10 24. The method of claim 17, wherein in said triturating comprises squeezing said clot through a mesh to separate said clot from said serum.

5 25. The method of claim 17, wherein said triturating comprises high speed centrifugation to separate said clot from said serum

15 26. The method of claim 17, wherein said serum and said platelet poor plasma are mixed in a ratio of 1:4 serum to platelet rich plasma.

10 27. The method of claim 17, wherein said serum and said platelet poor plasma are mixed in a ratio of 1:2 serum to platelet rich plasma.

20 28. The method of claim 17, wherein said serum and said platelet poor plasma are mixed in a ratio of 3:4 serum to platelet rich plasma.

 29. A method of producing an autologous bioadhesive sealant from a single whole blood sample, comprising:

15 forming an inactive platelet poor plasma from said whole blood sample;
25 dividing said inactive platelet poor plasma into a first and a second portion;
 reactivating said first portion of said inactive platelet poor plasma to form a clot;
 trituration said clot to obtain a serum comprising autologous thrombin; and
30 mixing said serum with said second portion of said inactive platelet poor plasma.

20 30. The method of claim 29, wherein said platelet poor plasma contains sodium citrate.

35 31. The method of claim 30, wherein said reactivation occurs with the addition of calcium ions.

 32. The method of claim 32, wherein said platelet poor plasma contains heparin.

25 33. The method of claim 29, wherein said reactivation occurs with the addition of heparinase.

40 34. The method of claim 30, wherein said platelet poor plasma containing sodium citrate is formed by:

 collecting said whole blood in a 9:1 ratio of whole blood to 3.8% sodium citrate solution forming a citrated blood mixture; and
45 centrifuging said citrated blood mixture to form a platelet poor plasma containing sodium citrate.

30 35. The method of claim 29, wherein said whole blood sample is obtained from an individual to whom said autologous bioadhesive sealant is to be applied.

50 36. The method of claim 29, wherein in said triturating comprises squeezing said clot

5 through a mesh to separate said clot from said serum.

37. The method of claim 29, wherein said triturating comprises high speed centrifugation to separate said clot from said serum

10 38. The method of claim 29, wherein said serum and said second portion of said platelet poor plasma are mixed in a ratio of 1:4 serum to platelet rich plasma.

5 39. The method of claim 29, wherein said serum and said second portion of said platelet poor plasma are mixed in a ratio of 1:2 serum to platelet rich plasma.

15 40. The method of claim 29, wherein said serum and said second portion of said platelet poor plasma are mixed in a ratio of 3:4 serum to platelet rich plasma.

10 41. A method of producing an autologous bioadhesive sealant from a single whole blood sample, comprising:

20 forming an inactive platelet rich plasma and an inactive platelet poor sample from said whole blood sample;

reactivating said inactive platelet poor plasma to form a clot;

25 15 triturating said clot to obtain a serum comprising autologous thrombin; and

mixing said serum with said platelet rich plasma.

42. The method of claim 41, wherein said platelet rich plasma and said platelet poor plasma each contains sodium citrate.

30 43. The method of claim 42, wherein said reactivating occurs with the addition of calcium ions.

44. The method of claim 41, wherein said platelet rich plasma and said platelet poor plasma each contains heparin.

35 45. The method of claim 44, wherein said reactivation occurs with the addition of heparinase.

25 46. The method of claim 42, wherein said platelet rich plasma and said platelet poor plasma containing sodium citrate are formed by:

40 collecting said whole blood in a 9:1 ratio of whole blood to 3.8% sodium citrate solution forming a citrated blood mixture; and

45 30 centrifuging said citrated blood mixture to form a platelet rich plasma and a platelet poor plasma each containing sodium citrate.

47. The method of claim 41, wherein said whole blood sample is obtained from an individual to whom said autologous bioadhesive sealant is to be applied.

50 48. The method of claim 41, wherein in said triturating comprises squeezing said clot through a mesh to separate said clot from said serum.

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49. The method of claim 41, wherein said triturating comprises high speed centrifugation to separate said clot from said serum

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50. The method of claim 41, wherein said serum and said platelet poor plasma are mixed in a ratio of 1:4 serum to platelet rich plasma.

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51. The method of claim 41, wherein said serum and said platelet poor plasma are mixed in a ratio of 1:2 serum to platelet rich plasma.

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52. The method of claim 41, wherein said serum and said platelet poor plasma are mixed in a ratio of 3:4 serum to platelet rich plasma.

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53. A method of producing an autologous bioadhesive sealant from a single whole blood sample, comprising:

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forming an inactive platelet rich plasma from said whole blood sample;

dividing said inactive platelet rich plasma into a first and a second portion;

adding human recombinant thromboplastin to said first platelet rich plasma portion

to produce thrombin; and

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combining said thrombin with said second inactive platelet rich plasma portion.

54. The method of claim 53, wherein said whole blood sample is obtained from an individual to whom said autologous bioadhesive sealant is to be applied.

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INTRINSIC PATHWAY

EXTRINSIC PATHWAY

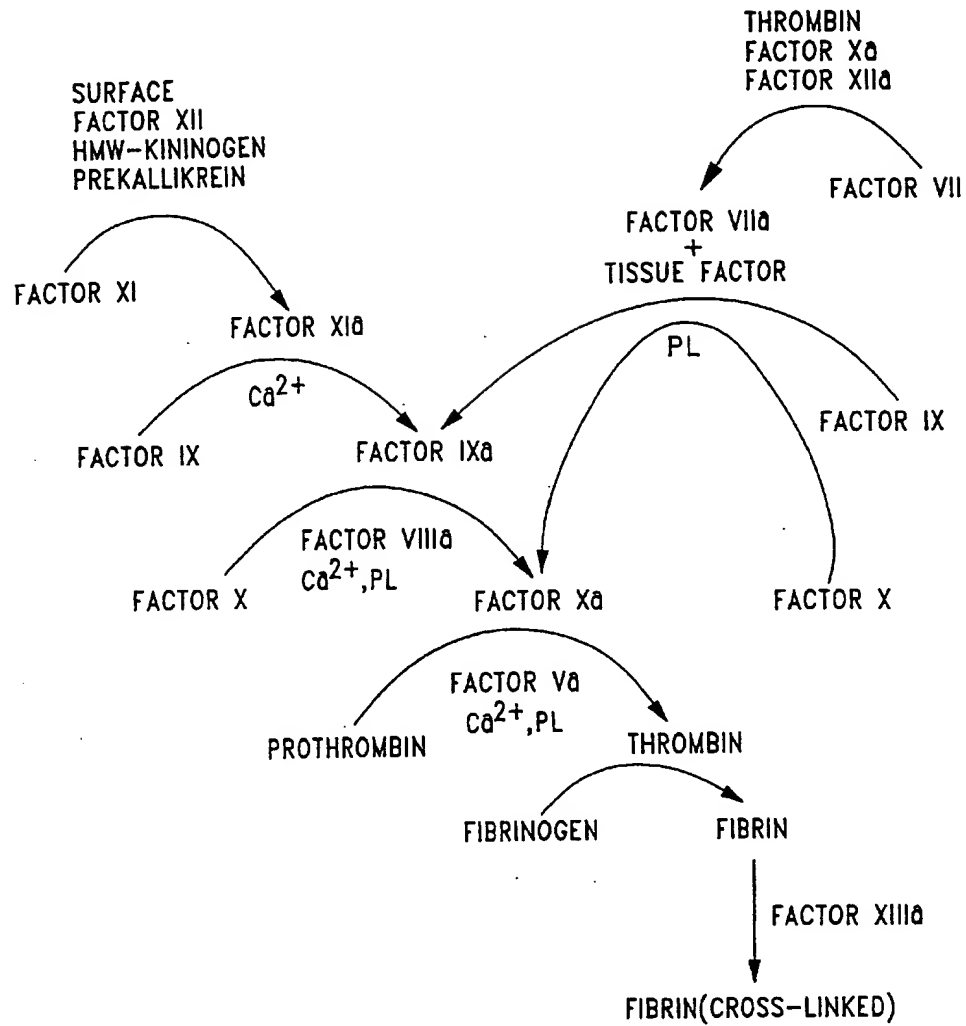


FIG. 1
(PRIOR ART)

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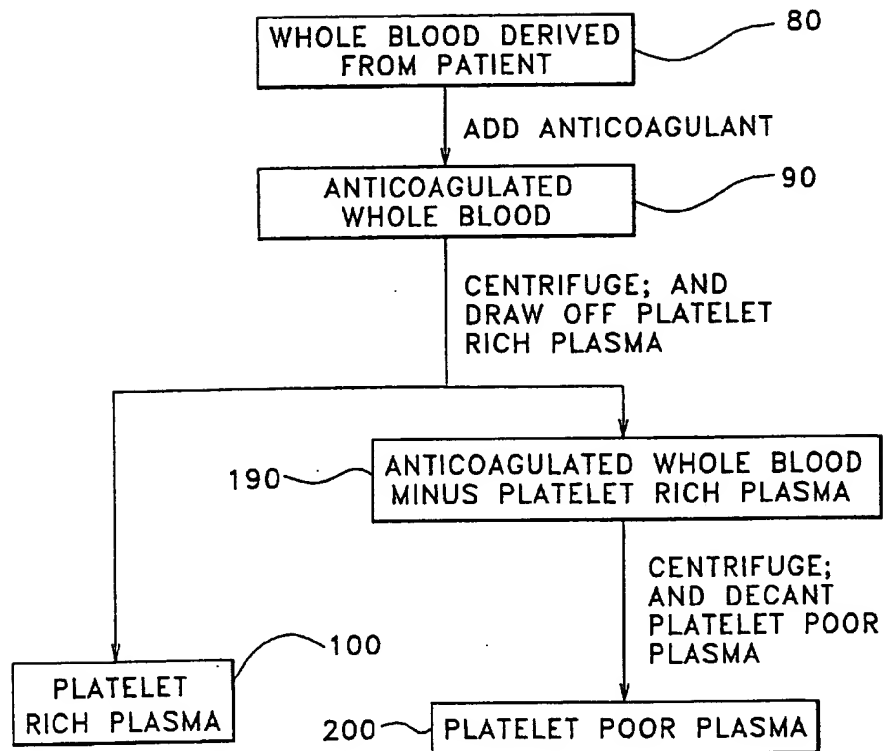


FIG. 2a

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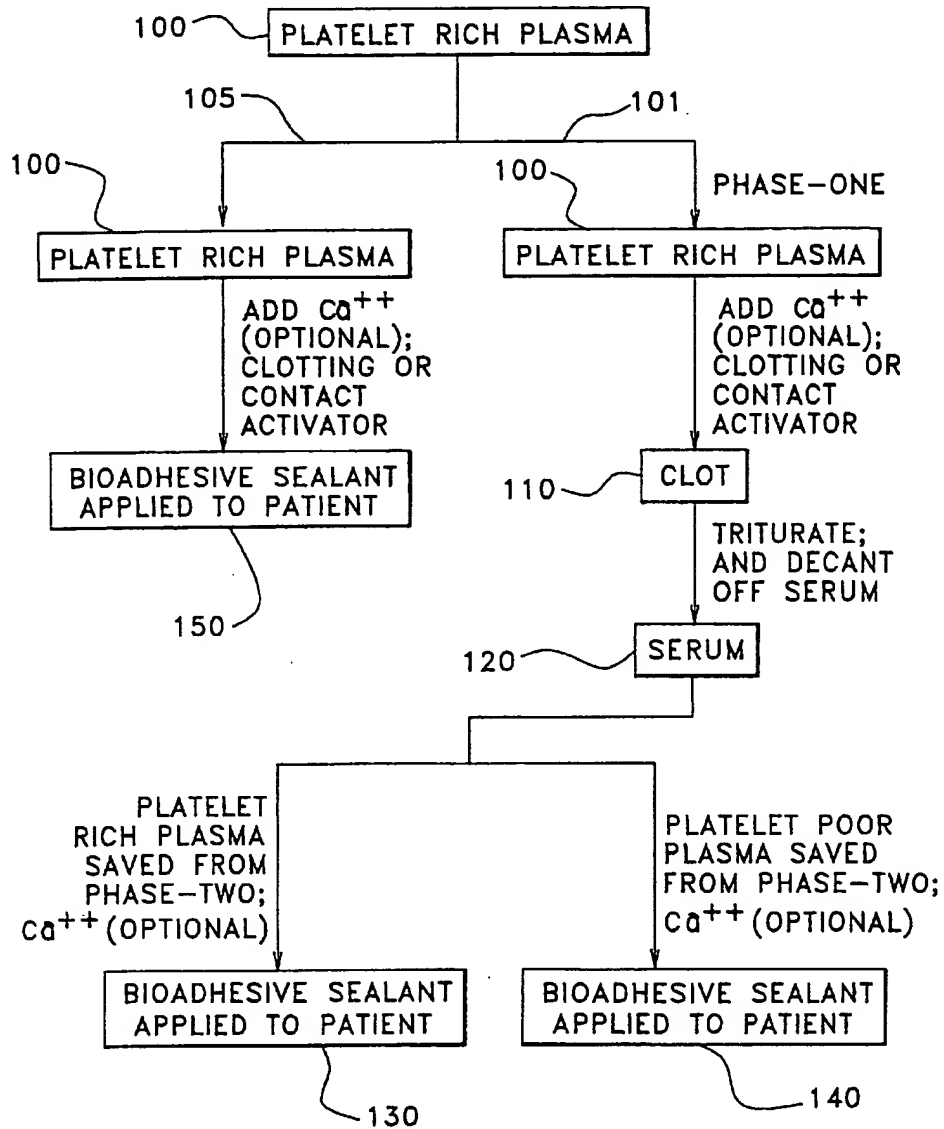


FIG. 2b

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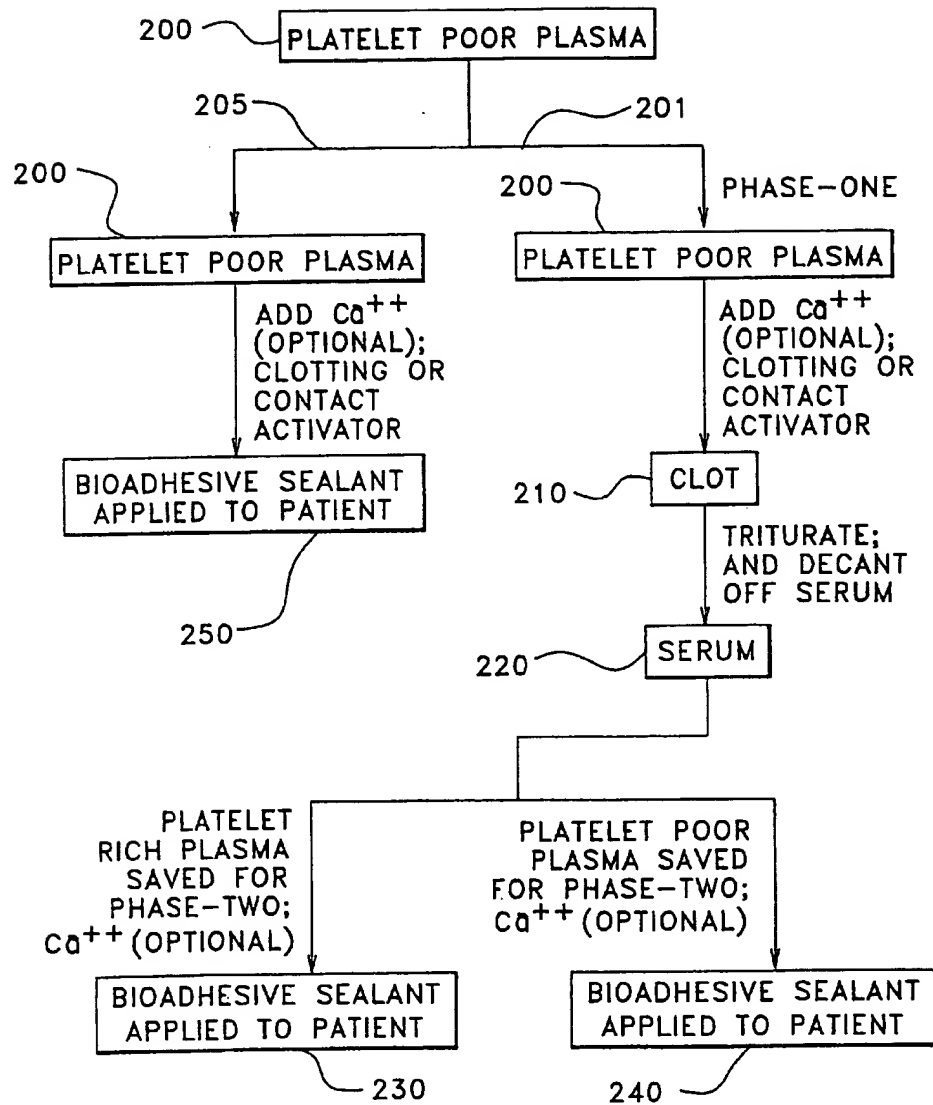


FIG. 2c

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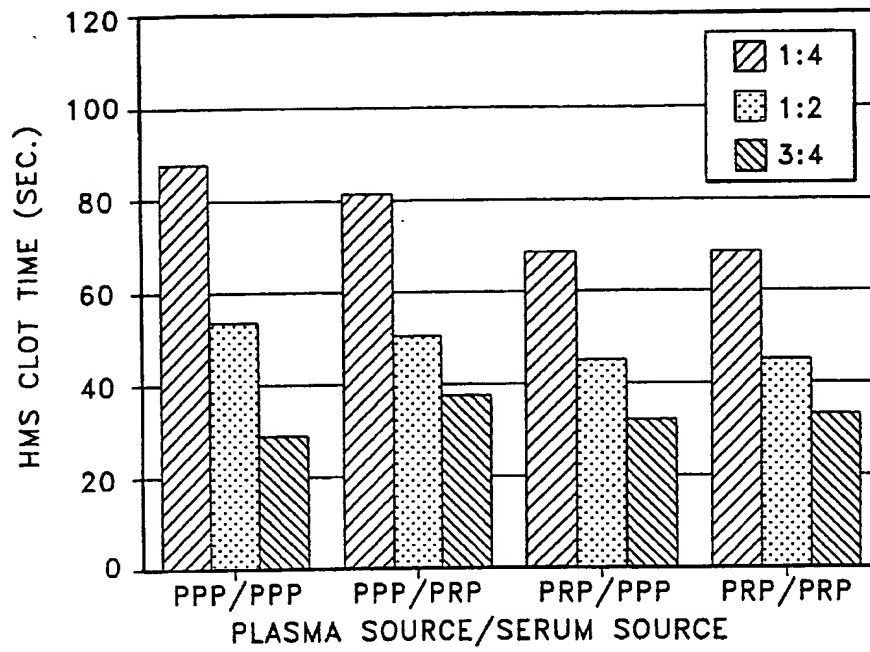


FIG. 3

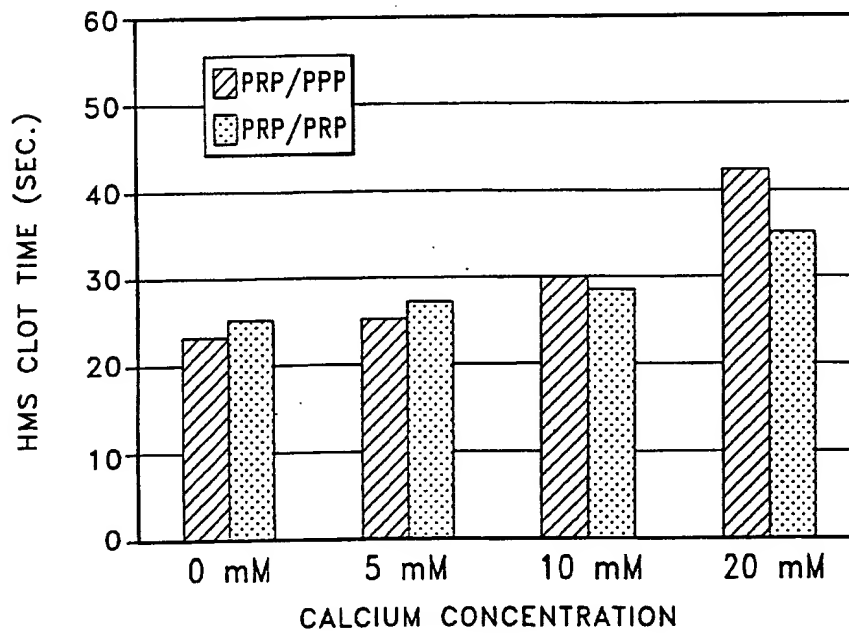


FIG. 6

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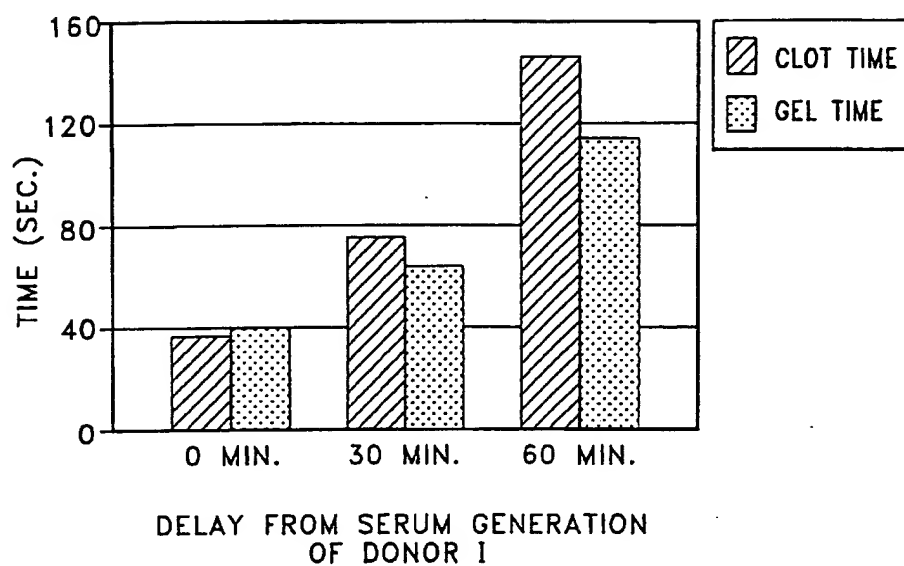


FIG. 4a

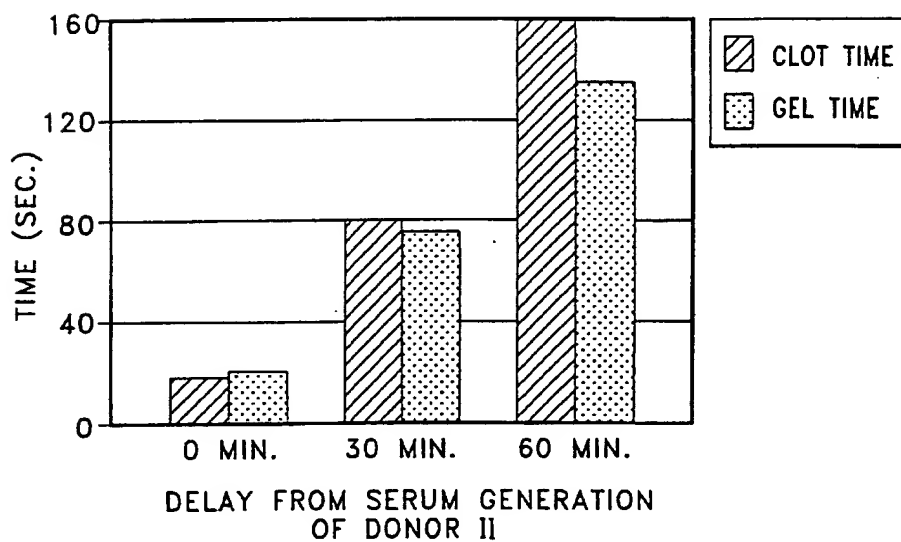


FIG. 4b

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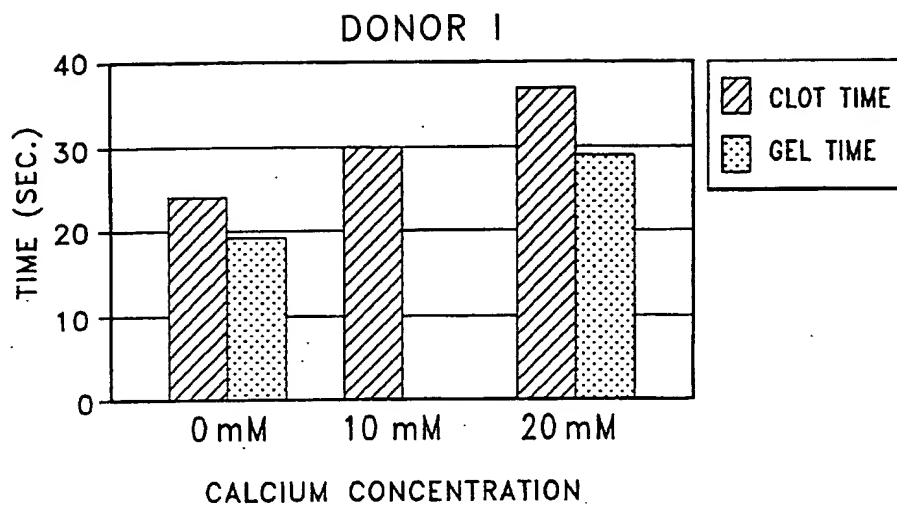


FIG. 5a

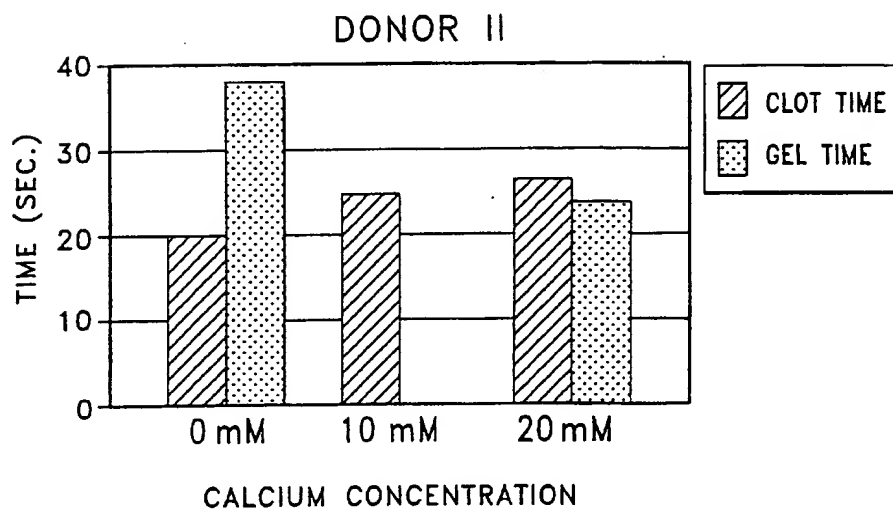


FIG. 5b

INTERNATIONAL SEARCH REPORT

International Application No.
PC./US 99/08550

A. CLASSIFICATION OF SUBJECT MATTER IPC 6 A61L25/00 A61K35/14		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) IPC 6 A61L		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used)		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 185 001 A (GALANAKIS DENNIS K) 9 February 1993 (1993-02-09) claims; example I	1-54
X	WO 91 09573 A (CRYOLIFE INC) 11 July 1991 (1991-07-11) claims	1-7, 10-54
X	DATABASE BIOSIS 'Online! BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US SUZUKI M. ET AL.: "CLINICAL APPLICATION OF THE FIBRIN ADHESIVE" XP002040697 abstract & OTOLARYNGOLOGY, vol. 56, no. 11, 1984, pages 949-953, TOKYO	8
-/-		
<input checked="" type="checkbox"/> Further documents are listed in the continuation of box C. <input checked="" type="checkbox"/> Patent family members are listed in annex.		
* Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. "&" document member of the same patent family		
Date of the actual completion of the international search		Date of mailing of the international search report
7 September 1999		17/09/1999
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl. Fax: (+31-70) 340-3018		Authorized officer
		ESPINOSA, M

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INTERNATIONAL SEARCH REPORT

International Application No
PC 1, US 99/08550

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 96 17871 A (PLASMASEAL CORP) 13 June 1996 (1996-06-13) page 48, line 10 - line 54; claims -----	1-7, 10-54
P,X	WO 97 29792 A (COHESION CORP ;SIERRA DAVID H (US)) 21 August 1997 (1997-08-21) the whole document -----	8
A	EP 0 443 724 A (BAXTER INT) 28 August 1991 (1991-08-28) column 1, line 41 - line 54 column 5, line 51 - line 58; claims -----	
A	FR 2 696 095 A (INOTEB) 1 April 1994 (1994-04-01) claims; examples -----	

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Form PCT/ISA210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No.

PCT, JS 99/08550

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5185001	A	09-02-1993	NONE
WO 9109573	A	11-07-1991	US 5030215 A 09-07-1991 AU 7220691 A 24-07-1991 CA 2072371 A 04-07-1991 EP 0509054 A 21-10-1992 JP 5504280 T 08-07-1993
WO 9617871	A	13-06-1996	US 5585007 A 17-12-1996 AU 696278 B 03-09-1998 AU 4286396 A 26-06-1996 BR 9506744 A 16-09-1997 CA 2182862 A 13-06-1996 EP 0743957 A 27-11-1996 JP 9509432 T 22-09-1997 NZ 297652 A 26-05-1997 US 5788662 A 04-08-1998
WO 9729792	A	21-08-1997	CA 2247133 A 21-08-1997 EP 0885020 A 23-12-1998
EP 0443724	A	28-08-1991	AT 177781 T 15-04-1999 DE 69130990 D 22-04-1999 JP 5227962 A 07-09-1993 US 5354682 A 11-10-1994
FR 2696095	A	01-04-1994	EP 0615454 A 21-09-1994 WO 9407548 A 14-04-1994 JP 7505076 T 08-06-1995 US 5589462 A 31-12-1996